Biotage® Plasmid Concentration Data

LB vs TB

Introduction

Mammalian protein production through transient transfection has become widely adopted in the early stages of therapeutic protein drug research and development. Recent advances in the transient transfection process and reagents have dramatically increased transfection efficiency. However, the transient nature of the process still demands a high level of plasmid DNA quantity and quality.

As large plasmid preps become more important, so does the optimization of scaling up the bacterial culture growth. It is important to get dense cultures for this application, with a high concentration of plasmids per cell, and to ensure culture to culture consistency. When optimizing culture growth, the selection of media is the first major consideration.

In this report, several common bacterial growth media were used to grow bacterial pellets specifically for processing in a maxi-scale plasmid prep. Pellets of similar size grown in each media are compared to estimate the amount of plasmid per cell grown in each culture.

Method

Reagents used in this study were purchased from VWR unless otherwise stated. Plasmid+® media was supplied by Thomson Instrument Company. The plasmid DNA used in this study is pUC cloned with eGFP. All cells were grown from a starter culture with ampicillin at a final concentration of 100 µg/mL, spun at 350 RMP at 37˚C in an incubator. Then, a 1:1000 dilution was taken and grown overnight for 16 hours, again with ampicillin at 100 µg/mL. Samples were centrifuged and supernatant decanted, then prepared and loaded on the AutoPlasmid MMG for an automated plasmid maxiprep.

Results and Discussion

Plasmid yields from the maxiprep of pellets grown from each media are displayed in Figure 1.

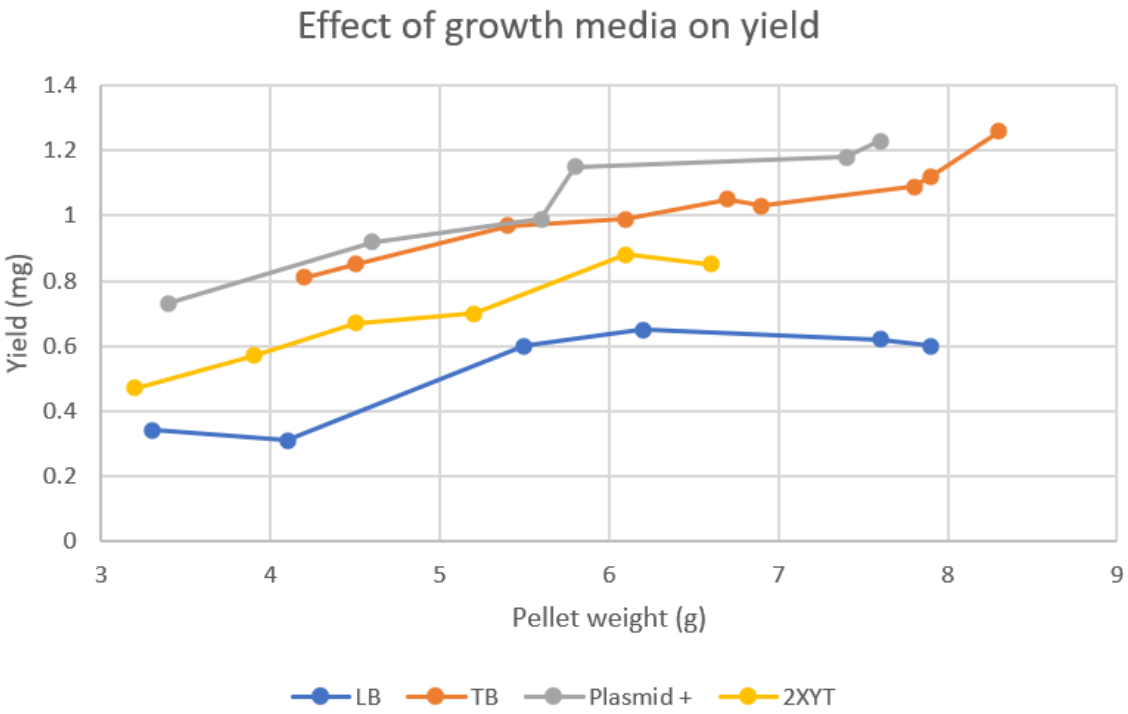

Figure 1. Pellets grown in several common bacterial culture medias are processed using an automated maxiprep system, the AutoPlasmid MMG. Yields are measured and analyzed to determine which media produces cultures with the highest plasmid count.

Cells grown in rich media, such as TB and Plasmid+®, have an average of 40-60% more plasmid per cell than cells grown in LB. 2XYT, another common rich media, performs better than LB and similarly to the other rich medias but the yield becomes more limited when scaling to much larger culture sizes and when grown for a certain period of time.

Also, each culture is denser when grown in TB or Plasmid+ which leads to a lower volume of media that is needed to grow the same sized pellets. Therefore, when aiming to achieve the maximum yield and efficiency of culture growth for the purpose of large-scale plasmid prep, a rich media such as TB or Plasmid+ is recommended.